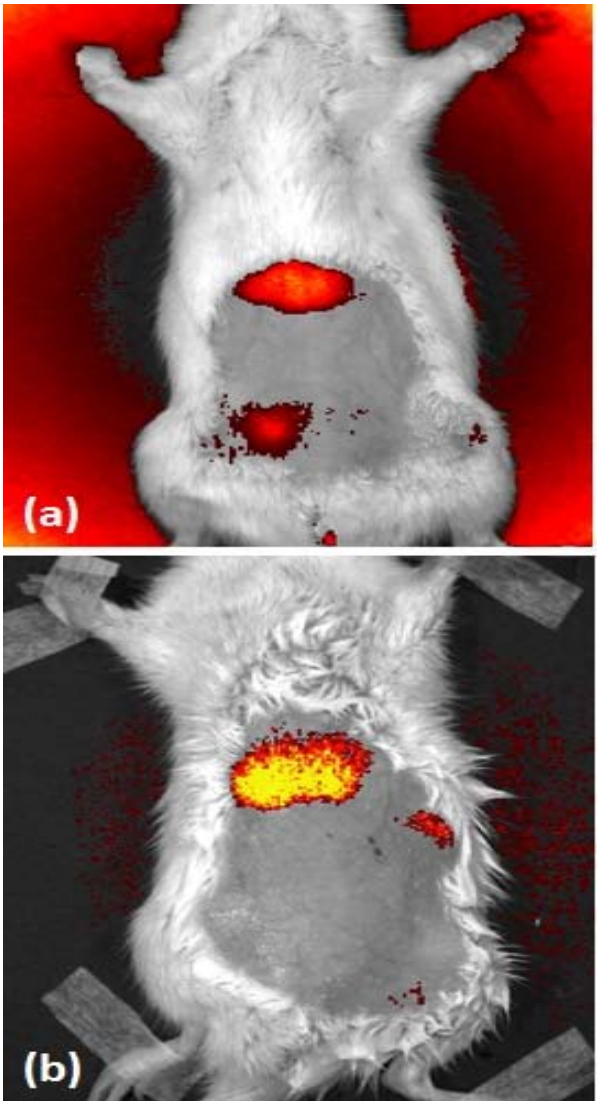
Material and Methods: A rat model was used to investigate a possible selective accumulation of circulating lymphocites to specific anatomical districts after radiation treatment focused to the urinary bladder. Eight Fisher rats were adoptively transferred with 4x107 VivoTag-750-labelled syngeneic primary splenocytes at two hours before the bladder irradiation. Two of eight rats were used as controls. Animals were transurethrally catheterized to allow contrast agent instillation. A kV cone beam computed tomography (CBCT) was acquired for each rat, to precisely deliver 6 MeV monofraction photon field. Rats were divided into three groups (n=2/group) receiving different levels of dose: 15, 20 and 25 Gy. A bolus thickness equal to 1cm was positioned on the rat skin surface in the pelvic region. Ultrasound images of the pelvic region were acquired at baseline, at 2, 4 and 6 days after irradiation to monitor thickness variations of the bladder wall tissue. In vivo fluorescent imaging was used to evaluated accumulation sites of labelled leukocytes.

Results: A significant increase in the bladder wall thickness was found 4 days after irradiation in animals treated with a dose equal to 25 Gy. A fluorescent signal, secondary to labelled splenocytes accumulation, emerged in the liver and lymph nodes of all adoptively transferred rats, 2 and 6 days after irradiation, as expected. A modest specific signal (30% increase) at the bladder level resulted only in two animals receiving the higher dose (Figure 1.a), when compared to the non-irradiated (Figure 1.b). No specific fluorescent signal was detected at the bladder levels in animals treated with 20 and 15 Gy.



Conclusion: The relocalization of peripheral leukocytes in the damaged tissue depends on the radiation dosage and it may be evaluated by means of a non-invasive imaging technique. Further analyses are currently ongoing.

EP-2054

Expression of DNA-PK in squamous cell lung cancer has gender differences and depends on smoking

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Purpose or Objective: Lung cancer is one of the most frequent and deadly types of cancer in Europe. Several aspects of non-small cell lung cancer (nsclc) in men and women continue to indicate potential male-female differences. Among these, higher treatment responses to current therapies in women are supposed, since women have better prognosis in any stage of the disease. In most stages of nsclc cytotoxic anti-cancer therapy (radiotherapy, chemotherapy) is used. It is known that treatment efficacy of cytotoxic anti-cancer therapy depends on tumor DNA-repair. Therefore, the aim of this study was to evaluate gender differences in the expression of DNA repair enzyme DNA protein kinase (DNA-PK).

Material and Methods: Surgically excised nsclc tissues (n=111, 50 adenocarcinomas, 61 squamous cell carcinomas) were examined for DNA-PK expression. After immunohistochemistry, the staining intensity of DNA-PK was quantified using an arbitrary score ranging from 0 (no staining) to 3 (strong signal). Also, the proportion (%) of DNA-PK positive (DNA-PK+) tumor cells was determined. All parameters were examined by 2 independent researchers in 10 randomly chosen microscopic fields (magnification x40).

Results: Immunohistochemical parameters were examined by 2 independent researchers whose results were in good accordance ($p<0.0005$). Staining intensities of DNA-PK and the proportion of DNA-PK+ tumor cells varied, being in the whole nsclc group 2.4±0.4 (mean±SD) and 86.3±9.1% respectively. There were no significant gender differences in adenocarcinoma. However, we detected significant differences among nsclc patients with squamous cell carcinoma. Both, DNA-PK staining intensity and the proportion of DNA-PK+ tumor cells were significantly higher in men than in women, 2.5±0.3 and 86.3±8.8% vs 2.1±0.6 and 79.6±11.9% respectively (DNA-PK intensity: $p<0.01$; DNA-PK+ proportion: $p=0,03$). Additionally, we found that in squamous cell carcinoma, the expression of DNA-PK depends on smoking and pack-years. There was a correlation between pack-years and DNA-PK intensity ($p=0.04$), as well as between pack-years and the proportion of DNA-PK+ tumor cells ($p=0.04$).

Conclusion: Expression of DNA-PK in squamous cell lung cancer has gender differences and depends on smoking. Significantly lower expression of tumor DNA-PK was found in women with this histological subtype of nsclc. Latter might be one of the reasons why cytotoxic anti-cancer therapy is more efficacious in women than in men. In further studies, the combination of DNA repair inhibitors and cytotoxic anti-cancer therapy should be tested.

EP-2055

Fibro-inflammatory circulating proteins as biomarkers for response in locally advanced rectal cancer

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Purpose or Objective: Selecting good responders after chemoradiation (CRT) for locally advanced rectal cancer (LARC) could lead to the omission of total mesorectal excision (TME) in patients with pathologic complete response (pCR). In the current study, we assessed the value of several blood biomarkers associated with the fibrotic response to CRT (IGF-1, IGFBP-2, HGF & GDF-15) as markers for general fibro-inflammatory response and as tumor response predictors in a group of 80 patients.

Material and Methods: ELISA analysis of IGF-1, IGFBP-2, HGF and GDF-15 was conducted on prospectively collected serum samples of 80 LARC patients on 3 time points (before, during, after CRT). The fibro-inflammatory response was scored on H&E sections of the resection specimen. Changes in concentration were analysed using a Kruskal-Wallis test. Correlation of concentration at each time point and the difference between these time points (Δ) with fibro-inflammatory response and tumor response (pCR and ypT0-1) were assessed using a Mann-Whitney-U test.

Results: Higher Growth Differentiation Factor 15 (GDF-15) concentration before CRT correlated with the presence of a fibro-inflammatory response ($p = 0.04$), but was not observed for the other proteins nor for GDF-15 at other time points.
General increase in GDF-15 concentration during treatment (median 0.81 ng/ml before, 2.16 ng/ml during, 2.37 ng/ml after CRT; $p < 0.0001$) was measured (Figure 1). Although no significant general concentration changes occurred for IGF-1, IGFBP-2 or HGF, we did find a correlation between the variation in expression of IGFBP-2 during treatment (ΔIGFBP-2 TP3-TP2) with tumor response (pCR $p = 0.02$; ypT0-1 $p = 0.02$). Other proteins did not correlate with tumor response.

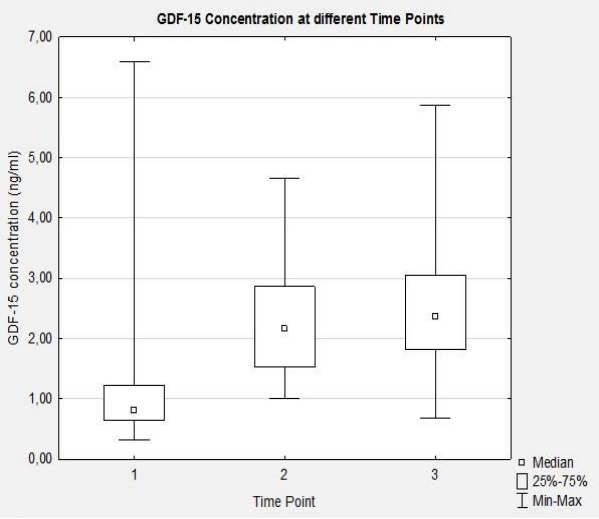

Figure 1. GDF-15 Concentration at different time points (TP) during treatment. (TP1: before CRT; TP2: during CRT; TP3: after CRT)

Conclusion: GDF-15 serum concentration increases during CRT for LARC and a higher concentration measured before start of treatment is correlated with the presence of a fibro-inflammatory response. These results suggest that GDF-15 could be used as an early predictor of fibro-inflammatory response and thereby indirectly as predictor for disease-free survival. This will be evaluated when follow-up data are available for this patient cohort.
The correlation of variation in expression of IGFBP-2 with tumor response (pCR and ypT0-1) opens a novel possibility for selecting good responders to CRT. We aim to combine these findings with imaging analyses (DW-MRI, PET) at different time points during treatment to develop a predictive model for selecting LARC patients in whom surgery could be omitted.

EP-2056
Preclinical investigation of hypoxia induced genes in different prostate cancer cell lines.
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Purpose or Objective: Hypoxia is a common feature in prostate cancer and is known to reduce the response to radiotherapy. Hypoxic modifiers can to a large extent overcome these obstacles, and a proper classification of tumors into hypoxic and non-hypoxic fractions is necessary. Previously our department has developed a gene profile consisting of 15 genes, which demonstrated prognostic and predictive impact for hypoxic modification in head and neck squamous cell carcinomas (HNSCC). In the current study we investigated the 15 gene profile in different prostate cancer cell lines.

Material and Methods: For the in vitro experiments the prostate cancer cell lines investigated were PC-3, DU-145, and LNCaP. Cell lines were cultured under normoxic (21% O2) or hypoxic conditions (0% O2) for 24 hours, totRNA was extracted and gene expression levels measured by qPCR. Individual reference genes were selected (PSMC4, TBP, NDFIP1) and applied in the normalization of the relative expression levels, together with the reference genes previously used in the HNSCC study. For in vivo experiments, the PC3 cell line was inoculated on the flank of female NMRI nu/nu mice, whereas the LNCaP and DU-145 cell lines were inoculated on the flank of severely immunocompromised CIEA/NOG mice. Two hypoxia-sensitive tracers (18F-FAZA and Pimonidazole) were administered in order to determine hypoxic and non-hypoxic regions on excised tumor sections. These regions were isolated by laser-assisted microdissection, after which totRNA was extracted and gene expression levels measured by qPCR.

Results: In the in vitro experiments, all prostate cancer cell lines had 14 of the 15 genes induced by hypoxia. The only discrepancy was ALDOA, which was not upregulated in the hypoxic cells. In vivo experiments are still ongoing but preliminary results from PC3 xenografts have been produced. These show a hypoxia induced upregulation in 10 out of the 15 genes, of which 4 were significantly upregulated (ADM, ANKRD37, FAM162A, and LOX).

Conclusion: In this study we investigated the 15 gene hypoxic profile in three different prostate cancer cell lines. A hypoxia dependent induction of genes was observed in both in vitro and in vivo experiments. From the performed experiments, and looking only at oxygen dependency, it appears that the gene profile could be suitable for prostate cancers as well as HNSCC.

EP-2057
Radiotoxicity prediction by gene expression profiling when simulating therapy in matched fibroblasts
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Purpose or Objective: Acute radiotoxicity might put a vital threat to the patient and may require interruption or